

SEARCH REQUEST FORM

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Art Unit: 1645 Phone Number 305-0481

Examiner #: 1645 Date: 9/10/01

Serial Number: 091424,244

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If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers; and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Method for Identifying Active Substances

Inventors (please provide full names): ANDREAS STEPHES GÜNTHER THUMM

JOHANNES POHLNER FRIEDRICH GÜTZ

Earliest Priority Filing Date: 9/1997

For Sequence Searches Only Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

B3,510

Gram positive bacteria (Staphylococci)
surface
polypeptides

Point of Contact:

Mary Hale

Technical Info. Specialist
CM1 12D16 Tel: 308-4258Enzymatic
Reporter assay

LPXTG etc terminal recognition motif
EnBPP portion
enzymatic activity of lipase
reporter

reporter - fluorescence spectroscopy
confocal fluorescence spect? interpeptidic pentaglycines
bond? murein

1430
14-01-31

Please search all the claims and the
inventors. Thank you

STAFF USE ONLY

Type of Search

Vendors and cost where applicable

Searcher: Mary

NA Sequence (#) STN 117,116

Searcher Phone #:

AA Sequence (#) Dialog

Searcher Location:

Structure (#) Questel/Orbit

Date Searcher Picked Up:

Bibliographic Dr.Link

Date Completed: 9/8

Litigation Lexis/Nexis

Searcher Prep & Review Time: 4

Fulltext Sequence Systems

Clerical Prep Time:

Patent Family WWW/Internet

Online Time: 9

Other Other (specify)

J. HINES
424244

=> fil reg

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TSCA INFORMATION NOW CURRENT THROUGH JANUARY 11, 2000

Please note that search-term pricing does apply when
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Structure search limits have been increased. See HELP SLIMIT
for details.

=> e gram positive bacteria/cn 5

E1	1	GRAM 474/CN
E2	1	GRAM BACTO/CN
E3	0 -->	GRAM POSITIVE BACTERIA/CN
E4	1	GRAM'S IODINE/CN
E5	1	GRAMAL 525/CN

=> e staphylococci/cn 5

E1	1	STAPHYLOCOCCAL NUCLEASE A/CN
E2	1	STAPHYLOCOCCAL PROTEINASE/CN
E3	0 -->	STAPHYLOCOCCCI/CN
E4	1	STAPHYLOCOCCIN/CN
E5	1	STAPHYLOCOCCIN 1580/CN

=> e lpxtg/sqep

E1	1	LPWYNHS/SQEP
E2	1	LPWYPSP/SQEP
E3	0 -->	LPXTG/SQEP
E4	1	LPY/SQEP
E5	1	LPY'BAL-OAA-OAA'/SQEP
E6	6	LPYA/SQEP
E7	1	LPYAAFLQDPIGWLFDRVAAQKIIISITRADVAHWRSKTADITASPNKRNTLIGFLAFFI GTSVIVLLELLDTHVKRPEDIEDTLQ/SQEP
E8	1	LPYAATALKLFAPTRLESVVLSTAIYKTYLSIRRGKSDGLLGTGIIAAMEIMSQNPVSV GIAVMLGVGAVAHHNAIEASEQKRTLLMKVFIKNFLDQAASDELVKESPEKIMALFEAV QTVGNPLRLVYHLYGVFYKGWGAKELAQRTAGRNLFTLIMFADAVELLGVDSSEGKVRQLSS NYILELLYKFRDSIKSSVRE/SQEP

Prepared by M. Hale 308-4258

E9 1 LPYACPVESCDRRFSRSDELTRHIRIH/SQEP
E10 1 LPYAG/SQEP
E11 1 LPYAGE/SQEP
E12 1 LPYDVPDYASLRS/SQEP

=> s lpxtg/sqsp

L1 1 LPXTG/SQSP

=> d sqide cbib abs

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2000 ACS
RN 246242-88-6 REGISTRY
CN Peptide, (Leu-Pro-Xaa-Thr-Gly-Xaa) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN PN: US5968763 SEQID: 1 claimed protein
FS PROTEIN SEQUENCE
SQL 6
NTE

type	----- location -----	description
uncommon	Aaa-3	-
uncommon	Aaa-6	-

SEQ 1 LPXTGX
=====

HITS AT: 1-5
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

REFERENCE 1: 131:283324 Method for screening inhibitors of the enzyme which cleaves the anchor of surface proteins from gram positive bacteria.
Fischetti, Vincent A.; Pancholi, Vijaykumar (Rockefeller University, USA).

U.S. US 5968763 A 19991019, 16 pp., Cont.-in-part of U. S. Ser. No. 319,540. (English). CODEN: USXXAM. APPLICATION: US 1997-819444 19970317. PRIORITY: US 1994-319540 19941007.

AB The invention relates to an enzyme which cleaves surface proteins of gram-pos. bacteria, to methods of detecting the enzyme, and methods of isolating the enzyme. In particular, the enzyme is isolated from a group A Streptococcus, and cleaves at the sequence LPXTGX. A method for screening putative inhibitors of the enzyme which cleaves the anchor region of surface proteins from gram-pos. bacteria is also disclosed. This enzyme and method provides a new antibiotic target for gram-pos. bacteria.

=> fil medi,capplus,biosis,embase,wpids;s (gram(1w)(pos. or positive)(w)bacter? or b3.510/ct) and staphylococc?
Prepared by M. Hale 308-4258

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	31.86	32.01
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-0.53	-0.53

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L2 2370 FILE MEDLINE
 L3 1915 FILE CAPLUS
 L4 1687 FILE BIOSIS
 L5 2859 FILE EMBASE
 L6 484 FILE WPIDS

TOTAL FOR ALL FILES
 L7 9315 (GRAM(1W) (POS. OR POSITIVE) (W) BACTER? OR B3.510/CT) AND
 STAPHYL
 OCOCC?

=> s 17 and (termin? recogn? or lpxtg or fnbpp or enzymat?(w) (reporter or
 activit?) or fluorescen? spectroscop? or confocal fluoresc? spectro?)

L8 18 FILE MEDLINE
 L9 12 FILE CAPLUS
 L10 17 FILE BIOSIS
 L11 16 FILE EMBASE
 L12 2 FILE WPIDS

TOTAL FOR ALL FILES
 L13 65 L7 AND (TERMIN? RECOGN? OR LPXTG OR FNBPP OR
 ENZYMAT? (W) (REPORT
 R OR ACTIVIT?) OR FLUORESCEN? SPECTROSCOP? OR CONFOCAL
 FLUORESC?
 SPECTRO?)

=> s 113 and (murein or interpeptide? or pentaglycine?)

L14 3 FILE MEDLINE
 Prepared by M. Hale 308-4258

L15 5 FILE CAPLUS
L16 3 FILE BIOSIS
L17 3 FILE EMBASE
L18 2 FILE WPIDS

TOTAL FOR ALL FILES

L19 16 L13 AND (MUREIN OR INTERPEPTIDE? OR PENTAGLYCINE?)

=> dup rem 119

PROCESSING COMPLETED FOR L19

L20 5 DUP REM L19 (11 DUPLICATES REMOVED)

=> d 1-5 cbib abs hit

L20 ANSWER 1 OF 5 MEDLINE

DUPLICATE 1

2000202059 Document Number: 20202059. Anchor structure of cell wall surface proteins in *Listeria monocytogenes*. Dhar G; Faull K F; Schneewind O. (Departments of Microbiology and Immunology and of Psychiatry and Biobehavioral Sciences, University of California Los Angeles School of Medicine, Los Angeles, California 90095, USA.) BIOCHEMISTRY, (2000 Apr 4)

39 (13) 3725-33. Journal code: A0G. ISSN: 0006-2960. Pub. country: United

States. Language: English.

AB Many surface proteins of **Gram-positive**

bacteria are anchored to the cell wall by a mechanism requiring a COOH-terminal sorting signal with a conserved **LPXTG** motif. In **Staphylococcus aureus**, surface proteins are cleaved between the threonine and the glycine of the **LPXTG** motif. The carboxyl of threonine is subsequently amide linked to the amino group of the **pentaglycine** cell wall crossbridge. Here we investigated the anchor structure of surface proteins in *Listeria monocytogenes*. A methionine and six histidines (MH(6)) were inserted upstream of the **LPXTG** motif of internalin A (InlA), a cell-wall-anchored surface protein of *L. monocytogenes*. The engineered protein InlA-MH(6)-Cws was found anchored in the bacterial cell wall. After peptidoglycan digestion with phage endolysin, InlA-MH(6)-Cws was purified by affinity chromatography. COOH-terminal peptides of InlA-MH(6)-Cws were obtained by cyanogen bromide cleavage followed by purification on a nickel-nitriloacetic acid column. Analysis of COOH-terminal peptides with Edman degradation and mass spectrometry revealed an amide linkage between the threonine of the cleaved **LPXTG** motif and the amino group of the m-diaminopimelic acid crossbridge within the listerial peptidoglycan. These results reveal that the cell wall anchoring of surface proteins in **Gram-positive bacteria** such as *S. aureus* and *L. monocytogenes* occurs by a universal mechanism.

AB Many surface proteins of **Gram-positive**

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Page 4

methionine and six histidines (MH(6)) were inserted upstream of the LPXTG motif of internalin A (InlA), a cell-wall-anchored surface protein of *L. monocytogenes*. The engineered protein InlA-MH(6)-Cws was found anchored in the bacterial cell wall. After peptidoglycan digestion with phage endolysin, InlA-MH(6)-Cws was purified by affinity chromatography. COOH-terminal peptides of InlA-MH(6)-Cws were obtained by cyanogen bromide cleavage followed by purification on a nickel-nitriloacetic acid column. Analysis of COOH-terminal peptides with Edman degradation and mass spectrometry revealed an amide linkage between the threonine of the cleaved LPXTG motif and the amino group of the m-diaminopimelic acid crossbridge within the listerial peptidoglycan. These results reveal that the cell wall anchoring of surface proteins in **Gram-positive bacteria** such as *S. aureus* and *L. monocytogenes* occurs by a universal mechanism.

L20 ANSWER 2 OF 5 CAPIUS COPYRIGHT 2000 ACS DUPLICATE 2
1999:234021 Document No. 130:277631 Method for identifying genes influencing

covalent attachment of proteins to **Gram-positive bacteria** surface. Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916900 A1 19990408, 58 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP6136 19980926.

AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the **Gram-pos. bacteria**; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6. Thus, a recombinant *Staphylococcus carnosus* clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of *S. hyicus* fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface,

but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the **murein** layer of *S. carnosus* were isolated and sequenced.

TI Method for identifying genes influencing covalent attachment of proteins to **Gram-positive bacteria** surface

AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of **Gram-**

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Page 5

pos. bacteria according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the **Gram-pos. bacteria**; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6. Thus, a recombinant **Staphylococcus carnosus** clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of *S. hyicus* fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the **murein** layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the **murein** layer of *S. carnosus* were isolated and sequenced.

ST **Gram pos bacteria** protein surface attachment gene screening; **Staphylococcus** lipase reporter **murein** attachment gene screening

IT Proteins (specific proteins and subclasses)
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fibronectin-binding, B, fusion protein with lipase, as reporter; method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT **Gram-positive bacteria** (Firmicutes)
Staphylococcus carnosus
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Proteins (general), biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Peptidoglycans
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**mureins**, attachment of proteins to; method for identifying genes influencing covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT DNA sequences
(of genomic fragments of **Staphylococcus carnosus** related to cell surface modification)

IT Plasmid vectors
(pTX30.DELTA.82, lipase precursor-fibronectin binding protein B fusion-encoding; method for identifying genes influencing covalent

attachment of proteins to **Gram-pos.**
bacteria surface)

IT Proteins (specific proteins and subclasses)
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(pathogenicity factors; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT Antibacterial agents
(screening for; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT 9001-62-1P, Lipase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion protein with fibronectin binding protein B, as reporter; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT 9033-39-0, Murein transpeptidase
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

IT 222837-02-7 222837-03-8 222837-04-9 222837-05-0 222837-06-1
222837-07-2 222837-08-3 222837-09-4 222837-10-7 222837-11-8
222837-12-9 222837-13-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; method for identifying genes influencing covalent attachment of proteins to **Gram-pos.**
bacteria surface)

L20 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3
1999:234015 Document No. 130:277630 Method for screening for agents which influence covalent attachment of proteins to **Gram-positive bacteria** surface. Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916894 A1 19990408, 51 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP6137 19980926.

AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the **Gram-pos. bacteria**. The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant *Staphylococcus carnosus* clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of *S. carnosus*. Prepared by M. Hale 308-4258 Page 7

hyicus fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium.

TI Method for screening for agents which influence covalent attachment of proteins to **Gram-positive bacteria** surface

AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of **Gram-pos. bacteria** according to the following steps: (1) prepn. of a **Gram-pos. bacteria** having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the **Gram-pos. bacteria**. The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant *Staphylococcus carnosus* clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of *S. hyicus* fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium.

ST **Gram pos bacteria** protein surface attachment antibiotic screening; **Staphylococcus** lipase reporter murein attachment sortase antibiotic screening

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RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fibronectin-binding, B, fusion protein with lipase, as reporter; method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT **Gram-positive bacteria** (Firmicutes)
Staphylococcus carnosus
(method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Proteins (general), biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(method for screening for agents which influence covalent attachment of proteins to **Gram-pos. bacteria** surface)

IT Peptidoglycans
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)

(mureins, attachment of proteins to; method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

IT Plasmid vectors
(pTX30.DELTA.82, lipase precursor-fibronectin binding protein B fusion-encoding; method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

IT Proteins (specific proteins and subclasses)
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(pathogenicity factors; method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

IT Antibacterial agents
(screening for; method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

IT 9001-62-1P, Lipase
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion protein with fibronectin binding protein B, as reporter; method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

IT 9033-39-0, Murein transpeptidase
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(method for screening for agents which influence covalent attachment of proteins to Gram-pos. bacteria surface)

L20 ANSWER 4 OF 5 MEDLINE DUPLICATE 4
1999269130 Document Number: 99269130. Multiple enzymatic activities of the murein hydrolase from staphylococcal phage phi11. Identification of a D-alanyl-glycine endopeptidase activity. Navarre W W; Ton-That H; Faull K F; Schneewind O. (Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, California 90095, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 28) 274 (22) 15847-56. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Bacteriophage muralytic enzymes degrade the cell wall envelope of staphylococci to release phage particles from the bacterial cytoplasm. Murein hydrolases of staphylococcal phages phi11, 80alpha, 187, Twort, and phiPVL harbor a central domain that displays sequence homology to known N-acetylmuramyl-L-alanyl amidases; however, their precise cleavage sites on the staphylococcal peptidoglycan have thus far not been determined. Here we examined the properties of the phi11 enzyme to hydrolyze either the staphylococcal cell wall or purified cell wall anchor structures attached to surface protein. Our results show that the phi11 enzyme has D-alanyl-glycyl endopeptidase as well as N-acetylmuramyl-L-alanyl amidase activity. Analysis of a deletion mutant lacking the amidase-homologous sequence, phi11(Delta181-381), revealed that the D-alanyl-glycyl endopeptidase activity is contained within the N-terminal 180 amino acid

residues of the polypeptide chain. Sequences similar to this N-terminal domain are found in the **murein** hydrolases of **staphylococcal** phages but not in those of phages that infect other **Gram-positive bacteria** such as **Listeria** or **Bacillus**.

TI Multiple enzymatic activities of the **murein** hydrolase from **staphylococcal** phage phi11. Identification of a D-alanyl-glycine endopeptidase activity.

AB Bacteriophage muralytic enzymes degrade the cell wall envelope of **staphylococci** to release phage particles from the bacterial cytoplasm. Murein hydrolases of **staphylococcal** phages phi11, 80alpha, 187, Twort, and phiPVL harbor a central domain that displays sequence homology to known N-acetylmuramyl-L-alanyl amidases; however, their precise cleavage sites on the **staphylococcal** peptidoglycan have thus far not been determined. Here we examined the properties of the phi11 enzyme to hydrolyze either the **staphylococcal** cell wall or purified cell wall anchor structures attached to surface protein. Our results show that the phi11 enzyme has D-alanyl-glycyl endopeptidase as well as N-acetylmuramyl-L-alanyl amidase activity. Analysis of a deletion mutant lacking the amidase-homologous sequence, phi11(Delta181-381), revealed that the D-alanyl-glycyl endopeptidase activity is contained within the N-terminal 180 amino acid residues of the polypeptide chain. Sequences similar to this N-terminal domain are found in the **murein** hydrolases of **staphylococcal** phages but not in those of phages that infect other **Gram-positive bacteria** such as **Listeria** or **Bacillus**.

CT Check Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Cell Wall: CH, chemistry
*Dipeptides: ME, metabolism
Endopeptidases: GE, genetics
*Endopeptidases: ME, metabolism
Hexosamines: AN, analysis
Molecular Sequence Data
Mutation
N-Acetylmuramoyl-L-alanine Amidase: CH, chemistry
*N-Acetylmuramoyl-L-alanine Amidase: ME, metabolism
Peptidoglycan: CH, chemistry
Recombinant Proteins: ME, metabolism
Sequence Alignment
Sequence Deletion
***Staphylococcus** Phages: EN, enzymology
Substrate Specificity
Viral Proteins: CH, chemistry
Viral Proteins: ME, metabolism

L20 ANSWER 5 OF 5 MEDLINE

DUPPLICATE 5

95215852 Document Number: 95215852. Structure of the cell wall anchor of surface proteins in **Staphylococcus** aureus. Schneewind O; Fowler A; Faull K F. (Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles 90024, USA.) SCIENCE, (1995 Apr 7) 268 (5207) 103-6. Journal code: UJ7. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Many surface proteins are anchored to the cell wall of **Gram-positive bacteria** and are involved in the pathogenesis
Prepared by M. Hale 308-4258

Page 10

of these organisms. A hybrid molecule was designed that, when expressed in **Staphylococcus aureus**, was anchored to the cell wall and could be released by controlled enzymatic digestion. By a combination of molecular biology and mass spectrometry techniques, the structure of the cell wall anchor of surface proteins in *S. aureus* was revealed. After cleavage of surface proteins between threonine and glycine of the conserved LPXTG motif, the carboxyl of threonine is amide-linked to the free amino group of the pentaglycine crossbridge in the **staphylococcal** cell wall.

TI Structure of the cell wall anchor of surface proteins in **Staphylococcus aureus**.

AB Many surface proteins are anchored to the cell wall of Gram-positive bacteria and are involved in the pathogenesis of these organisms. A hybrid molecule was designed that, when expressed in **Staphylococcus aureus**, was anchored to the cell wall and could be released by controlled enzymatic digestion. By a combination of molecular biology and mass spectrometry techniques, the structure of the cell wall anchor of surface proteins in *S. aureus* was revealed. After cleavage of surface proteins between threonine and glycine of the conserved LPXTG motif, the carboxyl of threonine is amide-linked to the free amino group of the pentaglycine crossbridge in the **staphylococcal** cell wall.

CT Check Tags: Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
*Bacterial Proteins: CH, chemistry
Base Sequence
Carrier Proteins: CH, chemistry
*Cell Wall: CH, chemistry
Chromatography, Affinity
Chromatography, High Pressure Liquid: MT, methods
Electrophoresis, Polyacrylamide Gel
*Membrane Proteins: CH, chemistry
Molecular Sequence Data
Recombinant Fusion Proteins: CH, chemistry
Staphylococcal Protein A: CH, chemistry
***Staphylococcus aureus: CH, chemistry**

CN 0 (maltose-binding protein); 0 (Bacterial Proteins); 0 (Carrier Proteins);
0 (Membrane Proteins); 0 (Recombinant Fusion Proteins); 0 (**Staphylococcal Protein A**)

=> s 17 and (murein or interpeptide? or pentaglycine?)

L21 7 FILE MEDLINE
L22 10 FILE CAPLUS
L23 5 FILE BIOSIS
L24 5 FILE EMBASE
L25 2 FILE WPIDS

TOTAL FOR ALL FILES

L26 29 L7 AND (MUREIN OR INTERPEPTIDE? OR PENTAGLYCINE?)

=> s 126 not 119

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Page 11

L27 4 FILE MEDLINE
L28 5 FILE CAPLUS
L29 2 FILE BIOSIS
L30 2 FILE EMBASE
L31 0 FILE WPIDS

TOTAL FOR ALL FILES
L32 13 L26 NOT L19

=> dup rem 132

PROCESSING COMPLETED FOR L32
L33 10 DUP REM L32 (3 DUPLICATES REMOVED)

=> d cbib abs 1-10

L33 ANSWER 1 OF 10 MEDLINE

2000179657 Document Number: 20179657. Functional analysis of heterologous
holin proteins in a lambdaDeltaS genetic background. Vukov N; Scherer S;
Hibbert E; Loessner M J. (Institut fur Mikrobiologie, Forschungszentrum
fur Milch und Lebensmittel, Weihenstephan, Technische Universitat
Munchen,

Weihenstephaner Berg 3, D-85350, Freising, Germany.) FEMS MICROBIOLOGY
LETTERS, (2000 Mar 15) 184 (2) 179-86. Journal code: FML. ISSN:
0378-1097. Pub. country: Netherlands. Language: English.

AB Holins are small hydrophobic proteins causing non-specific membrane
lesions at the end of bacteriophage multiplication, to promote access of
the murein hydrolase to their substrate. We have established a
lambdaDeltaS genetic system, which enables functional expression of
holins

from various phages in an isogenic phage lambda background, and allows
qualitative evaluation of their ability to support lysis of Escherichia
coli cells. Synthesis of Holins is under control of native lambda
transcription and translation initiation signals, and the
temperature-sensitive CI_{Its857} repressor. A number of different holins
were

tested in this study. The opposing action of phage lambda S105 and S107
holin variants in lysis timing could be confirmed, whereas we found
evidence for a functionally non-homologous dual translational start motif
in the Listeria phage Hol500 holin, i.e., the Hol500-96 polypeptide
starting at Met-1 revealed a more distinct lytic activity as compared to
the shorter product Hol500-93. The largest holin known, HolTW from a
Staphylococcus aureus phage, revealed an early lysis phenotype in
the lambdaDeltaSthf background, which conferred a plaque forming defect
due to premature lysis. Mutant analysis revealed that an altered
C-terminus and/or a V52L substitution were sufficient to delay lysis and
enable plaque formation. These results suggest that the extensively
charged HolTW C-terminus may be important in regulation of lysis timing.
The gene 17.5 product of E. coli phage T7 was found to support sudden,
saltatory cell lysis in the lambdaDeltaSthf background, which clearly
confirms its holin character. In conclusion, lambdaDeltaSthf offers a
useful genetic tool for studying the structure-function relationship of
the extremely heterogeneous group of holin protein orthologs.

L33 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
2000:414482 The fib locus in *Streptococcus pneumoniae* is required for peptidoglycan crosslinking and PBP-mediated β -lactam resistance. Weber, B.; Ehlert, K.; Diehl, A.; Reichmann, P.; Labischinski, H.; Hakenbeck, R. (Abt. Mikrobiol., Univ. Kaiserslautern, Kaiserslautern, Germany). FEMS Microbiol. Lett., 188(1), 81-85 (English) 2000. CODEN: FMLED7. ISSN: 0378-1097. Publisher: Elsevier Science B.V..

AB Penicillin resistance in pneumococci is mediated by modified penicillin-binding proteins (PBPs) that have decreased affinity to β -lactams. In high-level penicillin-resistant transformants of the lab. strain *Streptococcus pneumoniae* R6 contg. various combinations of low-affinity PBPs, disruption of the fib locus results in a collapse of PBP-mediated resistance. In addn., crosslinked muropeptides are highly reduced. The fib operon consists of two genes, fibA and fibB, homologous to *Staphylococcus aureus* femA/B which are also required for expression of methicillin resistance in this organism. FibA and FibB belong to a family of proteins of **Gram-pos.** bacteria involved in the formation of **interpeptide** bridges, thus representing interesting new targets for antimicrobial compds. for this group of pathogens.

L33 ANSWER 3 OF 10 MEDLINE
2000087512 Document Number: 20087512. Purification and partial characterization of a murein hydrolase, millericin B, produced by *Streptococcus milleri* NMSCC 061. Beukes M; Bierbaum G; Sahl H G; Hastings J W. (School of Molecular and Cellular Biosciences, University of Natal, Pietermaritzburg, Scottsville, South Africa.) APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2000 Jan) 66 (1) 23-8. Journal code: 6K6. ISSN: 0099-2240. Pub. country: United States. Language: English.

AB *Streptococcus milleri* NMSCC 061 was screened for antimicrobial substances and shown to produce a bacteriolytic cell wall hydrolase, termed millericin B. The enzyme was purified to homogeneity by a four-step purification procedure that consisted of ammonium sulfate precipitation followed by gel filtration, ultrafiltration, and ion-exchange chromatography. The yield following ion-exchange chromatography was 6.4%, with a greater-than-2,000-fold increase in specific activity. The molecular weight of the enzyme was 28,924 as determined by electrospray mass spectrometry. The amino acid sequences of both the N terminus of the enzyme (NH₂ SENDESLAMVSN) and an internal fragment which was generated by cyanogen bromide cleavage (NH₂ SIOTNAPWGL) were determined by automated Edman degradation. Millericin B displayed a broad spectrum of activity against **gram-positive bacteria** but was not active against *Bacillus subtilis* W23 or *Escherichia coli* ATCC 486 or against the producer strain itself. N-Dinitrophenyl derivatization and hydrazine hydrolysis of free amino and free carboxyl groups liberated from peptidoglycan digested with millericin B followed by thin-layer chromatography showed millericin B to be an endopeptidase with multiple activities. It cleaves the stem peptide at the N terminus of glutamic acid as well as the N terminus of the last residue in the **interpeptide** cross-link of susceptible strains.

L33 ANSWER 4 OF 10 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
93298584 EMBASE Document No.: 1993298584 Induction of release of tumor
Prepared by M. Hale 308-4258 Page 13

necrosis factor from human monocytes by **staphylococci** and **staphylococcal peptidoglycans**. Timmerman C.P.; Mattsson E.; Martinez-Martinez L.; De Graaf L.; Van Strijp J.A.G.; Verbrugh H.A.; Verhoef J.; Fleer A.. Eijkman-Winkler Med. Microbiol. Lab., Medical School, University of Utrecht, Utrecht, Netherlands. Infection and Immunity 61/10 (4167-4172) 1993.
ISSN: 0019-9567. CODEN: INFIBR. Pub. Country: United States. Language: English. Summary Language: English.

AB The role of cytokines in gram-positive infections is still relatively poorly defined. The purpose of this study was to establish whether or not intact **staphylococci** and purified peptidoglycans and peptidoglycan components derived from **staphylococci** are capable of stimulating the release of tumor necrosis factor (TNF) by human monocytes. We show here that intact **staphylococci** and purified peptidoglycans, isolated from three **Staphylococcus** epidermidis and three *S. aureus* strains, were indeed able to induce secretion of TNF by human monocytes in a concentration-dependent fashion. TNF release was detected by both enzyme immunoassay and the L929 fibroblast bioassay. In the enzyme immunoassay, a minimal concentration of peptidoglycan of 1 μ g/ml was required to detect TNF release by monocytes, whereas in the bioassay a peptidoglycan concentration of 10 μ g/ml was needed to detect a similar amount of TNF release. Peptidoglycan components such as the stem peptide, tetra- and **pentaglycine**, and muramyl dipeptide were unable to induce TNF release from human monocytes.

L33 ANSWER 5 OF 10 MEDLINE
90018830 Document Number: 90018830. Purification and peptidase activity of a bacteriolytic extracellular enzyme from *Pseudomonas aeruginosa*. Brito N; Falcon M A; Carnicero A; Gutierrez-Navarro A M; Mansito T B.
(Departamento de Microbiologia y Biología Celular, Facultad de Biología, Universidad de La Laguna, Tenerife, Spain..) RESEARCH IN MICROBIOLOGY, (1989 Feb) 140 (2) 125-37. Journal code: R6F. ISSN: 0923-2508. Pub. country: France.
Language: English.

AB A bacteriolytic enzyme excreted by *Pseudomonas aeruginosa* Paks I was purified: samples were found to be homogeneous by gel filtration chromatography, ion exchange chromatography using CM-cellulose, immunoelectrophoresis, PAGE and SDS-PAGE. The molecular weight of the lytic enzyme was estimated to be 15,000-19,000. The enzyme was active on Gram-positive bacteria with glycine-containing interpeptide bridges in their **murein** layers. In addition, this lytic enzyme showed peptidase activity catalysing the hydrolysis of **pentaglycine** peptides into tri- and diglycine peptides.

L33 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2000 ACS
1988:18213 Document No. 108:18213 Detection and characterization of a lytic proteinase, isolated from *Pseudomonas* lytica cultures, that lyses pathogenic organisms. Kulaev, I. S.; Severin, A. I.; Tauson, E. L.; Stepnaya, O. N. (Inst. Biokhim. Fiziol. Mikroorg., Pushchino, USSR). Vestn. Akad. Med. Nauk SSSR (7), 67-75 (Russian) 1987. CODEN: VAMNAQ.
ISSN: 0002-3027.

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AB P. lytica Cultured in a medium contg. 0.06% peptone, 0.01% yeast ext., and 0.5-2.5 mg/mL of killed **staphylococcal** cells was found to synthesize and secrete 1 bacteriolytic and 2 proteolytic enzymes. The bacteriolytic enzyme, which also displayed proteolytic activity, lysed all the pathogenic gram-pos. organisms examd., but was particularly active against **staphylococcal** cells. It hydrolyzed **Staphylococcus aureus** cell walls to liberate an N-terminal glycine and N-terminal alanine, indicating cleavage of the **pentaglycine** and peptidoglycan bonds and of the bond between muramic acid and alanine. Phys. properties of the bacteriolytic and proteolytic enzymes were detd.

L33 ANSWER 7 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS
1987:180503 Document No.: BR32:87630. IMMUNOELECTRON MICROSCOPIC STUDIES ON PEPTIDOGLYCAN FROM GRAM POSITIVE BACTERIA

SPECIFIC REACTIONS WITH THE GLYCAN MOIETY THE PENTAPEPTIDE SUBUNIT AND THE

INTERPEPTIDE BRIDGE. FRANKEN N; GOLECKI J R; SEIDL P H; ZWERENZ P; SCHLEIFER K H. BOEHRINGER MANNHEIM GMBH, FORSCHUNGSZENTRUM TUTZING, 8132 TUTZING, FRG.. SEIDL, P. H. AND K. H. SCHLEIFER (ED.). BIOLOGICAL PROPERTIES OF PEPTIDOGLYCAN; SECOND INTERNATIONAL WORKSHOP, MUNICH, WEST GERMANY, MAY 20-21, 1985. XIV+436P. WALTER DE GRUYTER: BERLIN, WEST GERMANY; NEW YORK, NEW YORK. ILLUS. (1986 (RECD 1987)) 0 (0), 135-144.

ISBN: 3-11-010737-6, 0-89925-262-1. Language: English.

L33 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2000 ACS
1984:433978 Document No. 101:33978 Nucleic acid hybridization studies and deoxyribonucleic acid base compositions of anaerobic, gram-positive cocci.

Huss, Volker A. R.; Festl, Herbert; Schleifer, Karl H. (Inst. Bot. Pharm. Biol., Univ. Erlangen, Erlangen, D-8520, Fed. Rep. Ger.). Int. J. Syst. Bacteriol., 34(2), 95-101 (English) 1984. CODEN: IJSBA8. ISSN: 0020-7713.

AB DNA-DNA reassocn. and DNA-rRNA cistron similarly studies showed that the anaerobic, gram-pos. cocci comprise a rather heterogeneous group of bacteria. The DNA-rRNA hybridization studies distinguished 7 groups. Groups 1 and 2 consisted of *Peptostreptococcus magnus* and *P. prevotii*, resp. *P. asaccharolyticus* ATCC 14963T (T = type strain) and *P. indolicus* ATCC 29427T formed a 3rd group, and *P. asaccharolyticus* DSM 20364

together with Hare group VIII strain NCTC 9820 formed group 4. *P. anaerobius* DSM 20357 was more closely related to *Eubacterium tenue* ATCC 25553T and *Clostridium lituseburense* ATCC 25759T than to any of the other species studied. *P. micros* Strains DSM 20468T and DSM 20367 together with

strains belonging to Hare group IX formed group 6, and group 7 consisted of *P. parvulus* 20469T. Strains of different Hare groups were all assigned to 1 of the 7 groups or to the genus **Staphylococcus** (Hare group VIIb strain NCTC 9819) or the genus **Streptococcus** (Hare group VIa strain NCTC 9806). The anaerobic cocci also have a diversity of **murein** structures. This report shows that strains belonging to the same species have different **murein** types.

L33 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2000 ACS

1982:16324 Document No. 96:16324 New insights into the three dimensional Prepared by M. Hale 308-4258 Page 15

arrangement of the cell walls of **staphylococci** and other **gram-positive bacteria**. Labischinski, H.; Barnickel, G.; Roenspeck, W.; Roth, K.; Giesbrecht, P. (Robert Koch-Inst., Berlin, Fed. Rep. Ger.). *Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Suppl.*, 10(*Staphylococci Staphylococcal Infect.*), 427-33 (English) 1981. CODEN: ZBMSDR.

AB On the basis of x-ray diffraction data on protected D-Glu-L-Lys dipeptide, stereochem. calcns., electron microscopy, and d. measurements, a model of **murein** is proposed for **gram-pos.** **bacteria**. The sugar chains in the **murein** network do not possess a chitin-like 2-fold screw axis, but lie tangentially in the plane of the cell wall and form a 3-4-sugar layered structure with a periodicity of .apprx.40-50 .ANG.. A mutual .apprx.60.degree. rotation of individual layers within a plywood-like arrangement is proposed. The relative order in the **murein** network is due to the radial arrangement, with respect to the sugar chains, of the peptide strands. Conformational energy calcns. suggest the most energetically favored peptide backbone conformation to be ring-like. This structure requires a fixed conformation of the .gamma.-bonded D-glutamic acid residue.

L33 ANSWER 10 OF 10 MEDLINE DUPLICATE 2
70183992 Document Number: 70183992. [The **murein** (peptidoglycan) types in **gram-positive bacteria**]. Die **Murein** (Pepidoglycan)-typen bei grampositiven Bakterien. Schleifer K H. ZENTRALBLATT FUR BAKTERIOLOGIE, PARASITENKUNDE, INFektionskrankheiten UND HYGIENE. 1. ABT. MEDIZINISCH-HYGIENISCHE BAKTERIOLOGIE, VIRUSFORSCHUNG UND PARASITOLOGIE. ORIGINALE, (1970) 212 (2) 443-51. Journal code: Y4Y. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

=> s strauss a?/au,in;s thumm g?/au,in;s pohlner j?/au,in;s gutz f?/au,in

'IN' IS NOT A VALID FIELD CODE
L34 394 FILE MEDLINE
L35 287 FILE CAPLUS
L36 481 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L37 282 FILE EMBASE
L38 60 FILE WPIDS

TOTAL FOR ALL FILES
L39 1504 STRAUSS A?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L40 4 FILE MEDLINE
L41 11 FILE CAPLUS
L42 4 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE

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Page 16

L43 4 FILE EMBASE
L44 16 FILE WPIDS

TOTAL FOR ALL FILES
L45 39 THUMM G?/AU, IN

'IN' IS NOT A VALID FIELD CODE
L46 21 FILE MEDLINE
L47 34 FILE CAPLUS
L48 23 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L49 18 FILE EMBASE
L50 11 FILE WPIDS

TOTAL FOR ALL FILES
L51 107 POHLNER J?/AU, IN

'IN' IS NOT A VALID FIELD CODE
L52 0 FILE MEDLINE
L53 0 FILE CAPLUS
L54 0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L55 0 FILE EMBASE
L56 0 FILE WPIDS

TOTAL FOR ALL FILES
L57 0 GUTZ F?/AU, IN

=> s 151 and 145 and 139

L58 0 FILE MEDLINE
L59 4 FILE CAPLUS
L60 0 FILE BIOSIS
L61 0 FILE EMBASE
L62 2 FILE WPIDS

TOTAL FOR ALL FILES
L63 6 L51 AND L45 AND L39

=> dup rem 163

PROCESSING COMPLETED FOR L63
L64 4 DUP REM L63 (2 DUPLICATES REMOVED)

=> d cbib abs 1-4

L64 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
1999:234021 Document No. 130:277631 Method for identifying genes
influencing
covalent attachment of proteins to Gram-positive bacteria surface.
Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz,
Friedrich (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916900
A1 19990408, 58 pp. DESIGNATED STATES: W: JP US; RW: AT, BE, CH CY,
Prepared by M. Hale 308-4258 Page 17

DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German).
CODEN:

PIXXD2. APPLICATION: WO 1998-EP6136 19980926.
AB The invention relates to a method for identifying genes which influence the covalent bonding of protein to the surface of Gram-pos. bacteria according to the following steps: (1) prepn. of a Gram-pos. bacteria having or forming at least one enzymic reporter substance which is or can be covalently bound to the bacterial surface, the activity of the enzyme differing depending on whether it is bound to the surface or free; (2) mutation of the genome of the Gram-pos. bacteria; (3) detn. of the enzymic activity of the reporter enzyme; (4) sepn. of bacteria with altered enzyme reporter activity; (5) isolation of the nucleic acids of these bacteria; (6) identification of nucleic acids contg. mutations; and (7) identification of genes using the nucleic acids isolated in step 6.

Thus, a recombinant *Staphylococcus carnosus* clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal sequence and lipase precursor-encoding gene of *S. hyicus* fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium. Using this system and transposon mutagenesis, 12 nucleic acids representing genes involved in the attachment of proteins to the murein layer of *S. carnosus* were isolated and sequenced.

L64 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
1999:234015 Document No. 130:277630 Method for screening for agents which influence covalent attachment of proteins to Gram-positive bacteria surface. **Strauss, Andreas; Thumm, Gunther; Pohlner, Johannes; Gotz, Friedrich** (Evotec Biosystems A.-G., Germany). PCT Int. Appl. WO 9916894 A1 19990408, 51 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE.
(German).

CODEN: PIIXD2. APPLICATION: WO 1998-EP6137 19980926.
AB The invention relates to a method for detg. active agents which influence the covalent bonding of protein to the surface of Gram-pos. bacteria according to the following steps: (1) prepn. of a Gram-pos. bacteria having or forming at least one enzymic reporter substance which is or can be covalently bound with the bacterial surface; (2) bringing the sample into contact with a possible active agent and; (3) detg. the enzymic activity of the reporter of the sample contg. the Gram-pos. bacteria.

The activity of the enzymic reporter differs depending on whether it is free or bound to the surface. Thus, a recombinant *Staphylococcus carnosus* clone contg. an expression vector with an inducible, chimeric reporter gene was prep'd. The chimeric reporter gene consisted of the signal

sequence and lipase precursor-encoding gene of *S. hyicus* fused to DNA encoding a C-terminal fragment of the fibronectin binding protein B (FnBPB) of *S. aureus*. Expression of the chimeric gene results in secretion of the fusion protein and cleavage of the signal peptide. Addnl., a sortase cleaves the fusion protein at the LPXTG recognition site

in the FnBPB C-terminus and attaches the fusion protein to the murein layer. A lipase fusion protein was developed which had no activity when covalently bound to the cell surface, but was only active when released into the culture medium.

L64 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2000 ACS

1999:355655 Document No. 131:1454 Lipase reporter assay detecting nucleic acids that code polypeptides involved in cell wall anchoring of surface proteins for application in antibiotics screening. **Pohlner, Johannes; Strauss, Andreas; Thumm, Gunther; Gotz, Friedrich** (Evotec Biosystems A.-G., Germany). Eur. Pat. Appl. EP 919631 A1 19990602, 21 pp.

DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL,

SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW.

APPLICATION: EP 1997-118755 19971029.

AB A reporter assay for identification of mutations affecting membrane anchoring of proteins that uses the secretion of a lipase from a *Staphylococcus carnosus* host as the reporter mechanism is described. The lipase carries the peptide LPETG, an example of the LPXGT peptides bound by fibronectin-binding protein B of *S. aureus* and mutations that affect the binding of the lipase and its attachment to the murein cell wall result in secretion of the enzyme into the medium where it can be detected. Lipase activity is assayed fluorometrically using a colorimetric substrate. Using this reporter assay, transposon mutagenesis

identified 10 genes involved in the membrane anchoring process.

L64 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2000 ACS

1999:292641 Document No. 130:293630 Antibiotic susceptibility screening by using a lipase reporter gene and reference mutants with cell wall anchoring inhibition of surface proteins. **Pohlner, Johannes; Strauss, Andreas; Thumm, Gunther; Gotz, Friedrich** (Evotec Biosystems AG, Germany). Eur. Pat. Appl. EP 913482 A1 19990506, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1997-118756 19971029.

AB The invention concerns the screening of biol. active substances, esp. antibiotics, that effect the anchoring of polypeptides to the surface of Gram-pos. bacteria using a series of procedures including the usage of microorganisms that express a hybrid polypeptide with a reporter substance

that when not anchored to the membrane possess a detectable property; contacting the microorganism with the potential active drug; and detecting

at least one of the properties of the reporter substance. The method applies ref. mutants that express a similar hybrid polypeptide contg. the reporter substance but are modified in order to inhibit the anchoring of the hybrid polypeptide on the cell wall. The hybrid polypeptide contains an N-terminal signal peptide, an enzyme or proenzyme, a linker peptide, a

and sequence expressing the cell wall anchoring motif LPXTG, a hydrophobic a charged sequence. Fluorometry can be used for the detection of the enzyme. Thus *Staphylococcus carnosus* was the host cell for the prodn. of the recombinant screening species; it was transfected with the assay plasmid pTX30.DELTA.82 that harbors a hybrid protein consisting of *Staphylococcus hyicus* lipase and the truncated C-terminal region of *Staphylococcus aureus* fibronectin binding protein B (FnBPB). The ref. *S. carnosus* mutants contained plasmids pTX30.DELTA.82.s and pTX30.DELTA.82.mem; both excreted the lipase contg. protein into the culture media. The *S. carnosus* mutants were used to screen erythromycin, tetracycline and chloramphenicol susceptibility using fluorescence correlation spectroscopy.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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